

SHEARMAN & STERLING

Menlo Park

1080 Marsh Road

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Telephone: (650) 838-3600

FAX NUMBER

(650) 838-3699

FAX COVER SHEET

March 28, 2002

Reference No. 01783/00102

Fax Recipient(s)				
Name	Firm	Location	Fax Number	Office Phone
Eva James	U.S.P.T.O.	Corrections Dept.	703.308.6672	703.305.8047

From

Name: Tina Patel
Telephone: 650.838.3753
Fax Number: 650.838.3699

Pages transmitted (including cover sheet):

Comments:

As per my voicemail, enclosed is a copy of the facsimile that was sent to you on September 26, 2001 as well as a copy of U.S. Patent No. 5,807,715.

Confidentiality Note: The information transmitted in this facsimile message is sent by an attorney or his/her agent, is intended to be confidential and for the use of only the individual or entity named above. If the recipient is a client, this message may also be for the purpose of rendering legal advice and thereby privileged. If the reader of this message is not the intended recipient, you are hereby notified that any retention, dissemination, distribution or copy of this telecopy is strictly prohibited. If you have received this facsimile in error, please immediately notify us by telephone and return the original message to us at the address above via the mail service (we will reimburse postage). Thank you.

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*** TX REPORT ***

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SHEARMAN & STERLING

01783-162

FAX NUMBER
(650) 838-3699

Menlo Park
1080 Marsh Road
Menlo Park, CA 94025-1022, USA
Telephone: (650) 838-3600

FAX COVER SHEET

September 26, 2001

Fax Recipients				
Name	Firm	Location	Fax Number	Office Phone
Eva James	USPTO		703.308.6672	

From

Name: Tina Patel
Telephone: 650.838.3753
Fax Number:

Pages transmitted (including cover sheet): 32

Comments:

Re: Correction of U.S. Patent No. 5,807,715

Miss James,

Per your request, enclosed is a copy of the transmittal letter and related papers that was sent to the PTO on June 25, 1999. As you will notice from the papers, two of the errors occur in the title and assignee fields of the patent and both of these errors are believed to be the responsibility of the PTO. As I previously discussed with you and Miss Watson, I understand that because of these errors, a new ribbon grant of this patent should be issued once all of the errors are

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Patentees : Sherie L. Morrison
Leonard A. Herzenberg
Vernon T. Oi

Patent No. : 5,807,715

Issued : September 15, 1998

On Application
Serial No. : 08/266,154

For : METHODS AND TRANSFORMED MAMMALIAN
LYMPHOCYTIC CELLS FOR PRODUCING
FUNCTIONAL ANTIGEN-BINDING PROTEIN
INCLUDING CHIMERIC IMMUNOGLOBULIN
AND FRAGMENTS

Group Art Unit : 1642

Examiner : Julie E. Reeves, Ph.D.

Hon. Asst. Commissioner for Patents
Attn: Certificate of Correction Branch
Washington, D.C. 20231

REQUEST UNDER 37 C.F.R. § 1.322 AND § 1.323
FOR ISSUANCE OF CORRECTED COPY OF PATENT

Sir:

Enclosed are 8 sheets (in duplicate) of Form PTO-1050 listing corrected printing errors that have been found in the above-identified patent. None of the corrections add new matter.

Two of the listed errors, believed to be the responsibility of the Patent and Trademark Office, are of such import that the patentees and their assignees respectfully request that in lieu of a certificate of correction, the Patent and Trademark Office issue a corrected original ribbon grant of the patent, and a corrected electronic copy of the patent.

First, one assignee, The Trustees of Columbia University, New York, New York was omitted. Both assignees, The Board of Trustees of The Leland Stanford Junior University and The Trustees of Columbia University, were listed in Part B-Issue Fee Transmittal (and attached sheet), copies of which are attached as Exhibit A. Also attached, as Exhibit B, is the Notice of Recordation of Assignment Document listing Columbia University as an assignee.

Second, the title as printed on the issued patent is in error. The correct title is:

METHODS AND TRANSFORMED MAMMALIAN LYMPHOCYTIC
CELLS FOR PRODUCING FUNCTIONAL ANTIGEN-BINDING
PROTEIN INCLUDING CHIMERIC IMMUNOGLOBULIN AND
FRAGMENTS,

as amended in the Second Amendment After Allowance Pursuant to 37 C.F.R. § 1.312(a), filed May 18, 1998, and entered by the Examiner on May 22, 1998. Copies of the Amendment and the Examiner's Response to Rule 312 Communication are attached as Exhibit C and D, respectively.

The two errors described above are those that motivate the present request for issuance of a new patent. Form PTO-1050 lists additional corrections, most of which report clear typographical errors. However, two corrections are requested in the claims, meriting additional discussion.

First, claim 41, as corrected, would depend from claim 37, rather than claim 39. Applicants believe that this correction is necessitated by incorrect renumbering by the Patent and Trademark Office of amended claim 136, filed in the Second Amendment After Allowance.

Second, the requested deletion of two separate uses of the word "are" in each of claims 41 and 42 corrects grammatical difficulties present in the claims as issued,


and does not add new matter or change the scope of the claims.

Some of the corrected errors were made by the applicants. Accordingly, enclosed herewith is a check for \$100.00 in payment of the fee set forth in 37 C.F.R. § 1.20(a). We also request 15 printed copies of the patent, if the corrected patent is issued; a check in the amount of \$45.00 to cover the cost of the copies is enclosed. The Commissioner is hereby authorized to charge any additional fees due, or credit any overpayment, in connection with these respective Requests to Deposit Account No. 06-1075. A duplicate copy of this Request for Issuance of Corrected Copy of Patent is enclosed herewith.

If any questions arise from the foregoing, the Applicants invite an inquiry to the undersigned by telephone at (650) 617-4011.

Respectfully submitted,

Dated: June 25, 1999


Vicki S. Veenker
Registration No. 34,269
Attorney for Patentees

c/o FISH & NEAVE
1251 Avenue of the Americas
New York, New York 10020
(650) 617-4000

Attachments:

I hereby Certify that this
Correspondence is being
Deposited with the U.S.
Postal Service as First
Class Mail in an Envelope
Addressed to: ASSISTANT
COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231, ON

June 25, 1999

MARTHA D. JOHNSON


Signature of Person Signing

- Exhibit A
Part B-Issue Fee Transmittal, Form PTOL-85B, and
attached sheet, applic. ser. no. 08/266,154
- Exhibit B
Notice of Recordation of Assignment Document,
applic. ser. no. 07/090,669

Attachments (cont.):

- Exhibit C
Pages 1 - 8 of Second Amendment After Allowance,
May 18, 1998, applic. ser. no. 08/266,154
- Exhibit D
Response to Rule 312 Communication entering Second
Amendment After Allowance, applic. ser.
no.08/266,154

Enclosures:

- PTO-1050 (8 sheets, in duplicate)
- Check in the amount of \$100.00, for providing a certificate of correction under 37.C.F.R. § 1.323.
- Check in the amount of \$45.00, to cover the fee for 15 printed copies of the corrected patent.

PART B—ISSUE FEE TRANSMITTAL

Complete and mail this form, together with applicable fees, to:

Box ISSUE FEE
Assistant Commissioner for Patents
Washington, D.C. 20231.

MAILING INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE. Blocks 1 through 4 should be completed where appropriate. All further correspondence including the Issue Fee Receipt, the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Legibly mark-up with any corrections or use Block 1)

HM31/G218

VICKI S VEENKER
FISH & NEAVE
1251 AVENUE OF THE AMERICAS
NEW YORK NY 10020

Note: The certificate of mailing below can only be used for domestic mailings of the Issue Fee Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing.

Certificate of Mailing

I hereby certify that this Issue Fee Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Box Issue Fee address above on the date indicated below.

MARTHA D JOHNSON (Depositor's name)

Mullowne (Signature)

MAY 18, 1998 (Date)

APPLICATION NO.	FILING DATE	TOTAL CLAIMS	EXAMINER AND GROUP ART UNIT	DATE MAILED :
087266, 154	06/27/94	050	REEVES, J	1642 02/18/98
First Named Applicant				

TITLE OF INVENTION

SHERIE L.

METHODS FOR PRODUCING FUNCTIONAL IMMUNOGLOBIN, INCLUDING CHIMERIC IMMUNOGLOBIN, IN TRANSFORMED MAMMALIAN LYMPHOCYTIC CELLS
(AS AMENDED)

ATTY'S DOCKET NO. -	CLASS-SUBCLASS	BATCH NO.	APPLN. TYPE	SMALL ENTITY	FEE DUE	DATE DUE
435-069-600	A72	UTILITY	NO		\$1320.00	05/18/98

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363). Use of PTO form(s) and Customer Number are recommended, but not required.

☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.

☐ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47) attached.

2. For printing on the patent front page, list (1) the names of up to 3 registered patent attorneys or agents OR, alternatively, (2) the name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1. Fish & Neave
2. Vicki S. Veenker
3. Edward F. Mullowne

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)
PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. Inclusion of assignee data is only appropriate when an assignment has been previously submitted to the PTO or is being submitted under separate cover. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE* The Board of Trustees of
The Leland Stanford Junior University

(B) RESIDENCE (CITY & STATE OR COUNTRY)

Stanford, California

Please check the appropriate assignee category indicated below (will not be printed on the patent)

☐ Individual ☒ corporation or other private group entity ☐ government

4a. The following fees are enclosed (make check payable to Commissioner of Patents and Trademarks):

☒ Issue Fee
☒ Advance Order - # of Copies 15

4b. The following fees or deficiency in these fees should be charged to:

DEPOSIT ACCOUNT NUMBER 06-1075
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☐ Issue Fee
☐ Advance Order - # of Copies

The COMMISSIONER OF PATENTS AND TRADEMARKS IS requested to apply the Issue Fee to the application identified above.

(Authorized Signature)

Vicki S. Veenker 34,269

(Date)

5/18/98

NOTE: The Issue Fee will not be accepted from anyone other than the applicant; a registered attorney or agent; or the assignee or other party in interest as shown by the records of the Patent and Trademark Office.

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*See attached sheet for second

TRANSMIT THIS FORM WITH FEE

PTOL-558 (REV. 11-94)

Additional Assignee Name and Residence Data to be Printed on the Patent

(A) Name of Assignee The Trustees of Columbia University

(B) Residence: (City & State or Country)
New York, New York

☐ Individual ☒ Corporation or other private group entity ☐ Government

03/28/02 09:57 FAX 650 839 3699

EXHIBIT B

Notice of Recordation of Assignment Document,
applic. ser. no. 07/090,669

03/28/02 09:57 FAX 650 839 3699



UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office

ASSISTANT SECRETARY AND COMMISSIONER
OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

ST-1-2-1/2402

TO: LEYDIG, VOIT & MAYER
350 CAMBRIDGE AVENUE
SUITE 200
PALO ALTO, CA 94306

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LEYDIG, VOIT & MAYER
PALO ALTO

UNITED STATES PATENT AND TRADEMARK OFFICE
NOTICE OF RECORDATION OF ASSIGNMENT DOCUMENT

THE ENCLOSED DOCUMENT HAS BEEN RECORDED BY THE ASSIGNMENT DIVISION OF
THE U.S. PATENT AND TRADEMARK OFFICE. A COMPLETE MICROFILM COPY IS
- AVAILABLE AT THE U.S. PATENT AND TRADEMARK OFFICE ON THE REEL AND FRAME -
NUMBER REFERENCED BELOW. A DIGEST OF THE DOCUMENT HAS ALSO BEEN MADE
AND APPEARS IN THE OFFICE'S RECORDS AS SHOWN:

ASSIGNOR: 001 MORRISON, SHERIE L.

DOC DATE: 10/17/87

RECORDATION DATE: 11/16/87 NUMBER OF PAGES 002 REEL/FRAME 4830/0958

DIGEST: ASSIGNMENT OF ASSIGNORS INTEREST

ASSIGNEE: 501 TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK,
THE, BROADWAY AND WEST 116TH STREET, NEW YORK, NEW YORK,
10027, A CORP. OF NEW YORK.

SERIAL NUMBER 7-090669 FILING DATE 08/28/87

PATENT NUMBER ISSUE DATE 00/00/00

TITLE OF INVENTION: CHIMERIC RECEPTORS BY DNA SPLICING AND EXPRESSION

INVENTOR: 001 MORRISON, SHERIE L.

- INVENTOR: 002 HERZENBERG, LEONARD A.

INVENTOR: 003 OI, VERNON T.

EXHIBIT C

Second Amendment After Allowance,
May 18, 1998, applic. ser. no. 08/266,154

08/266,154

BD1 CIP FWC IV

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Sherie L. Morrison et al.
Serial No. : 08/266,154
Filed : June 27, 1994
For : METHODS FOR PRODUCING FUNCTIONAL
IMMUNOGLOBULIN, INCLUDING CHIMERIC
IMMUNOGLOBULIN, IN TRANSFORMED
MAMMALIAN LYMPHOCYTIC CELLS

Group Art Unit : 1806

Examiner : Julie E. Reeves, Ph.D.

Hon. Assistant Commissioner
for Patents
Washington, D.C. 20231

May 18, 1998

SECOND AMENDMENT AFTER ALLOWANCE
PURSUANT TO 37 C.F.R. 1.312(a)

Sir:

Applicants request approval under Rule 312(a) for entry of the following amendment without withdrawing the case from issue.

IN THE TITLE

Please replace the current title with:

METHODS AND TRANSFORMED MAMMALIAN LYMPHOCYTIC CELLS FOR
PRODUCING FUNCTIONAL ANTIGEN-BINDING PROTEIN INCLUDING
CHIMERIC IMMUNOGLOBULIN AND FRAGMENTS

IN THE CLAIMS

Please amend claims 129-131 and 134-137 as follows:

129. A method as recited in claim 126 wherein [each] the first chain comprises a constant region.

130. A method as recited in claim 126 wherein the heavy and light chain variable domains are from [domain is found in] a first mammalian species and the heavy and light chain constant domains are from [domain is found in] a second mammalian species, said second mammalian species being other than the first mammalian species.

131. A method as recited in claim 129 wherein the heavy and light chain variable domains are from [domain is found in] a first mammalian species and the heavy and light chain constant regions are from [region is found in] a second mammalian species, said second mammalian species being other than the first mammalian species.

134. A method as recited in claim 132 wherein prior to step (a) the cell endogenously produces an immunoglobulin light chain or an immunoglobulin heavy chain, [which endogenously-produced heavy chain is not secreted in a form capable of specifically binding to antigen,] but not both.

135. A method as recited in claim 132 wherein [each] the first chain [has] comprises a constant region.

136. A method as recited in claim 132 wherein the antigen-binding protein [immunoglobulin] comprises the heavy and light chain variable domains are from [domain found in] a first mammalian species and comprises the heavy and light chain constant domains are from [domain found in] a second mammalian species, said second mammalian species being other than the first mammalian species.

137. A method as recited in claim 135 wherein the antigen-binding protein [immunoglobulin] comprises the heavy and light chain variable domains are from [domain found in] a first mammalian species and comprises the heavy and light chain constant regions are from [region found in] a second mammalian species, said second mammalian species being other than the first mammalian species.

Please add the following claims 138-157.

138. A method for producing a functional antigen-binding protein comprising

- i) a first chain comprising an immunoglobulin heavy chain variable domain and an immunoglobulin heavy chain constant domain and
- ii) a second chain comprising an immunoglobulin light chain variable domain and an immunoglobulin light chain constant domain,

wherein the method comprises the steps of:

- (a) maintaining in a nutrient medium a transformed mammalian lymphocytic cell, said cell having been transfected with a first DNA molecule coding for the first chain of the protein and a second DNA molecule coding for the second chain of the protein;

(b) expressing from said cell the first and second chains functionally assembled together to form said protein which is then secreted in a form capable of binding antigen; and

(c) recovering said antigen-binding protein,
wherein prior to being transfected, the cell does not express a functional immunoglobulin capable of specifically binding antigen.

139. A method as recited in claim 138 wherein prior to step (a) the cell does not endogenously produce any immunoglobulin chains.

140. A method as recited in claim 138 wherein prior to step (a) the cell endogenously produces an immunoglobulin light chain or an immunoglobulin heavy chain, but not both.

141. A method as recited in claim 138 wherein the first chain comprises a constant region.

142. A method as recited in claim 138 wherein the heavy and light chain variable domains are from a first mammalian species and the heavy and light chain constant domains are from a second mammalian species, said second mammalian species being other than the first mammalian species.

143. A method as recited in claim 141 wherein the heavy and light chain variable domains are from a first mammalian species and the heavy and light chain constant regions are from a second mammalian species, said second mammalian species being other than the first mammalian species.

144. A transformed mammalian lymphocytic cell producing a functional antigen-binding protein comprising

- i) a first chain comprising an immunoglobulin heavy chain variable domain and an immunoglobulin heavy chain constant domain and
- ii) a second chain comprising an immunoglobulin light chain variable domain and an immunoglobulin light chain constant domain,

wherein the transformed mammalian lymphocytic cell comprises:

- (a) a first exogenous DNA molecule coding for the first chain of the protein; and
- (b) a second exogenous DNA molecule, said second DNA molecule coding for the second chain of the protein;

wherein without the exogenous DNA molecules the cell does not express a functional antigen-binding protein.

145. A transformed mammalian lymphocytic cell as recited in claim 144 wherein without the exogenous DNA molecules the cell does not endogenously produce any immunoglobulin chains.

146. A transformed mammalian lymphocytic cell as recited in claim 144 wherein without the exogenous DNA molecules the cell endogenously produces an immunoglobulin light chain or an immunoglobulin heavy chain, but not both.

147. A transformed mammalian lymphocytic cell as recited in claim 144 wherein the first chain comprises a constant region.

148. A transformed mammalian lymphocytic cell as recited in claim 144 wherein the heavy and light chain variable domains are from a first mammalian species and the heavy and light chain constant domains are from a second mammalian species, said second mammalian species being other than the first mammalian species.

149. A transformed mammalian lymphocytic cell as recited in claim 147 wherein the heavy and light chain variable domains are from a first mammalian species and the heavy and light chain constant regions are from a second mammalian species, said second mammalian species being other than the first mammalian species.

150. A transformed mammalian lymphocytic cell producing a functional antigen-binding protein comprising

- i) a first chain comprising an immunoglobulin heavy chain variable domain and an immunoglobulin heavy chain constant domain and
- ii) a second chain comprising an immunoglobulin light chain variable domain and an immunoglobulin light chain constant domain,

wherein the transformed mammalian lymphocytic cell comprises:

a plasmid comprising a first exogenous DNA molecule coding for the first chain of the protein and a second exogenous DNA molecule coding for the second chain of the protein; and

wherein without the exogenous DNA molecules the cell does not express a functional antigen-binding protein.

151. A transformed mammalian lymphocytic cell as recited in claim 150 wherein without the exogenous DNA molecules the cell does not endogenously produce any immunoglobulin chains.

152. A transformed mammalian lymphocytic cell as recited in claim 150 wherein without the exogenous DNA molecules the cell endogenously produces an immunoglobulin light chain or an immunoglobulin heavy chain, but not both.

153. A transformed mammalian lymphocytic cell as recited in claim 150 wherein the first chain comprises a constant region.

154. A transformed mammalian lymphocytic cell as recited in claim 150 wherein the heavy and light chain variable domains are from a first mammalian species and the heavy and light chain constant domains are from a second mammalian species, said second mammalian species being other than the first mammalian species.

155. A transformed mammalian lymphocytic cell as recited in claim 153 wherein the heavy and light chain variable domains are from a first mammalian species and the heavy and light chain constant regions are from a second mammalian species, said second mammalian species being other than the first mammalian species.

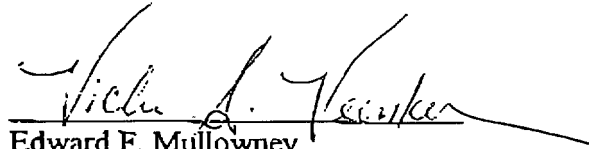
156. A transformed mammalian lymphocytic cell produced by steps (a) and (b) of claim 126.

157. A transformed mammalian lymphocytic cell produced by step (a) of claim 132.

REMARKS

Applicants believe claims 138-157 are fully supported by the specification and respectfully request their entry and allowance.

Respectfully submitted,



Edward F. Mullenney
Registration No. 27,459
Vicki S. Veenker
Registration No. 34,269
Attorney for Applicants

c/o FISH & NEAVE
1251 Avenue of the Americas
New York, New York 10020-1104
Tel.: (212) 596-9000

03/28/02 09:58 FAX 650 839 3699

EXHIBIT D

Response to Rule 312 Communication entering
Second Amendment After Allowance,
applic. ser. no.08/266,154


**UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office**

 Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
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08/266,154

06/27/94

MORRISON

S

HD101PF-W0111

EXAMINER

HM21/0542

REEVES, J

ART UNIT

PAPER NUMBER

VICKI S. VEENKER

FISH & NEAVE

1291 AVENUE OF THE AMERICANS

NEW YORK NY 10020

1442

DATE MAILED:

MAY 22/98

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

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MAY 29 1998

 FISH & NEAVE - PATENT DEPT.
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SENT TO
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BY A. Wilson

**Response to Rule 312
Communication**Application No.
08/266,154Applicant(s)
Morrison et alExaminer
Julie E. Reeves, Ph.D.Group Art Unit
1642

The petition filed on _____ under 37 CFR 1.312(b) is granted. The paper has been forwarded to the examiner for consideration on the merits.

X The amendment filed on May 18, 1998 under 37 CFR 1.312 has been considered, and has been:

X entered.

entered as directed to matters of form not affecting the scope of the invention (Order 3311).

disapproved. See explanation below.

entered in part. See explanation below.


LILA FEISEE
SUPERVISORY PATENT EXAMINER

PTO/SB/44 (10-96)

Approved for use through 6/30/99. OMB 0651-0033

Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

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(Also Form PTO-1050)

UNITED STATES PATENT AND TRADEMARK OFFICE CERTIFICATE OF CORRECTION

PATENT NO : 5,807,715

DATED : September 15, 1998

INVENTOR(S): S.L. Morrison, Herzenberg, L.A., and Oi, V.T.

It is certified that error appears in the above-identified patent and that said Letters Patent are hereby corrected as shown below:

In the Title [54], delete "LYMPHOCYTE" and replace with --LYMPHOCYTIC--; after "IMMUNOGLOBULIN", add --AND FRAGMENTS--.

In the Inventors [75], delete "Scarsdale, N.Y." and replace with --Los Angeles--; delete "Menlo Park" and replace with --Mountain View--; delete "both" and replace with --all--.

In the Assignee [73], delete "Assignee" and replace with --Assignees--; after "Calif." add --, and The Trustees of Columbia University, New York, N.Y.--.

In References Cited [56], after "Seno et al 1983 Nucleic", delete "Acid" and replace with --Acids--; after "Research 11(3)", delete ";" and replace with --:--.

In References Cited [56], after "Dolby et al 1980 PNAS 77(10)" add --:--.

Column 1, line 2, delete "LYMPHOCYTE" and replace with --LYMPHOCYTIC--.

Column 1, line 5, after "IMMUNOGLOBULIN" add --AND FRAGMENTS--.

Column 1, line 16, add the paragraph --The work described herein was supported in part by grants from the National Institutes of Health (NIH), including AI-00408, AI-08917, CA-04681, and CA-16858. The United States Government has certain rights in the invention.--

Column 1, line 19, delete "1."

MAILING ADDRESS OF SENDER: Fish & Neave
1251 Avenue of the Americas
New York, NY 10020

PATENT NO. 5,807,715

No. of additional copies



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PTO/SB/44 (10-96)

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(Also Form PTO-1050)

UNITED STATES PATENT AND TRADEMARK OFFICE CERTIFICATE OF CORRECTION

PATENT NO : 5,807,715

DATED : September 15, 1998

INVENTOR(S): S.L. Morrison, Herzenberg, L.A., and Oi, V.T.

It is certified that error appears in the above-identified patent and that said Letters Patent are hereby corrected as shown below:

Column 1, line 41, delete "2."

Column 1, line 61, after "functional", add --,--; after "assembled", add --,--.

Column 1, line 66, delete "mouse-" and replace with --mouse:-

Column 1, line 67, delete ":".

Column 3, line 26, delete "prepar" and replace with --prepara--.

Column 3, line 32, delete "CDNA" and replace with --cDNA--.

Column 4, line 45, delete "CDNA" and replace with --cDNA--.

Column 4, line 52, delete "CDNA" and replace with --cDNA--.

Column 4, line 59, delete "CDNA" and replace with --cDNA--.

Column 4, line 65, delete "Joining" and replace with --joining--.

Column 8, line 2, after "immunization", add --,--.

Column 8, line 21, delete "VH" and replace with --V_H--.

Column 8, line 22, delete "Vk" and replace with --V_κ--
(Greek letter kappa subscripted).

MAILING ADDRESS OF SENDER: Fish & Neave
1251 Avenue of the Americas
New York, NY 10020

PATENT NO. 5,807,715

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PTO/SB/44 (10-96)

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UNITED STATES PATENT AND TRADEMARK OFFICE CERTIFICATE OF CORRECTION

PATENT NO : 5,807,715

DATED : September 15, 1998

INVENTOR(S): S.L. Morrison, Herzenberg, L.A., and Oi, V.T.

It is certified that error appears in the above-identified patent and that said Letters Patent are hereby corrected as shown below:

Column 8, line 24, delete "VH" and replace with --V_H--.

Column 8, line 29, delete "V_K" and replace with --V_κ--
(Greek letter kappa subscripted).

Column 8, line 31, delete "J_K" and replace with --J_κ--
(Greek letter kappa subscripted); delete "C_K" and replace with --C_κ--
(Greek letter kappa subscripted).

Column 9, line 9, delete "Five" and replace with --Fine--;
delete "Chamicals" and replace with --Chemicals--.

Column 9, line 19, delete "VH-VL" and replace with --V_H-V_L--.

Column 9, line 57, delete "H2L2" and replace with --H₂L₂--.

Column 9, line 67, delete "K" and replace with --κ--; delete
"antiodies" and replace with --antibodies--.

Column 10, line 5, after "expected" add --,--.

Column 10, line 10, delete "VH" and replace with --V_H--;
delete "V_K" and replace with --V_κ-- (Greek letter kappa
subscripted).

Column 10, line 14, delete "mouse: human" and replace with --
mouse:human--.

Column 10, line 22, delete "VH" and replace with --V_H--;
delete "V_K" and replace with --V_κ-- (Greek letter kappa
subscripted).

MAILING ADDRESS OF SENDER: Fish & Neave

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INVENTOR(S): S.L. Morrison, Herzenberg, L.A., and Oi, V.T.

It is certified that error appears in the above-identified patent and that said Letters Patent are hereby corrected as shown below:

Column 10, line 36, delete "Mr" and replace with --M_r--.

Column 10, line 42, delete "Mr" and replace with --M_r--.

Column 10, line 54, delete "hybri-doma-" and replace with --hybridoma--.

Column 11, line 5, delete "V_κ" and replace with --V_κ--
(Greek letter kappa subscripted).

Column 11, line 10, delete "mouse: human" and replace with --mouse:human--.

Column 11, line 21, delete "use" and replace with --used--.

Column 11, line 35, delete "IgG₁, IgG₂, IgG₃, IgG₄, rabbit IgG and mouse IgG₃," and replace with --IgG₁, IgG₂, IgG₃, IgG₄, rabbit IgG and mouse IgG₃--.

Column 11, line 37, delete "(delta)" and replace with --Δ--
(Greek letter capital delta).

Column 11, line 41, delete "V_κ" and replace with --V_κ--
(Greek letter kappa subscripted).

Column 11, line 43, after "Ann" add --.---.

Column 11, line 44, delete "V_κ" and replace with --V_κ--
(Greek letter kappa subscripted).

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PATENT NO : 5,807,715
 DATED : September 15, 1998
 INVENTOR(S): S.L. Morrison, Herzenberg, L.A., and Oi, V.T.

It is certified that error appears in the above-identified patent and that said Letters Patent are hereby corrected as shown below:

Column 11, line 60, delete "pure, >95%" and replace with --pure (>95%)--.

Column 11, line 63, delete "mouse-human" and replace with --mouse:human--.

Column 11, line 64, delete "IgG₃" and replace with --IgG3--.

Column 12, line 3, delete "IgG₁" and replace with --IgG1--.

Column 12, line 10, delete "affinity purified" and replace with --affinity-purified--.

Column 12, line 22, delete "labelling" and replace with --labeling--.

Column 12, line 26, delete "pp." and replace with --p.--.

Column 12, line 31, after "Kornfeld" add --,--.

Column 12, line 32, delete "Wigzell Proc," and replace with --Wigzell, Proc.--.

Column 12, line 33, after "et al." add --,--.

Column 12, line 42, delete "IgG₁-IgG_{2a}" and replace with --IgG1-IgG2a--.

Column 12, line 64, delete "IgG₁" and replace with --IgG1--;
 delete "IgG_{2a}" and replace with --IgG2a--.

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INVENTOR(S): S.L. Morrison, Herzenberg, L.A., and Oi, V.T.

It is certified that error appears in the above-identified patent and that said Letters Patent are hereby corrected as shown below:

Column 12, line 65, delete "IgG_{2a}" and replace with --IgG_{2a}--;
delete "IgG₁" and replace with --IgG₁--.

Column 13, line 4, delete "T₄" and replace with --T₄--.

Column 13, line 43, after "Gilbert" add --,--.

Column 13, line 65, delete "Dangi" and replace with --Dangl--.

Column 14, line 24, delete "bateria" and replace with --bacteria--.

Column 14, line 41, delete "C." and replace with --C--.

Column 14, line 53, delete "labelled" and replace with --labeled--.

Column 15, line 5, delete "IgG₁" and replace with --IgG₁--;
delete "IgG_{2a}" and replace with --IgG_{2a}--.

Column 15, line 25, delete "pHGXL_{C_v}aB" and replace with --pHGXL_{C_v}B--.

Column 15, line 27, delete "Trp*" and replace with --TRP*--.

Column 15, line 39, delete "Trp*" and replace with --TRP*--.

Column 15, line 44, delete "IgG₁" and replace with --IgG₁--.

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INVENTOR(S): S.L. Morrison, Herzenberg, L.A., and Oi, V.T.

It is certified that error appears in the above-identified patent and that said Letters Patent are hereby corrected as shown below:

Column 15, line 45, delete "IgG_{2a}" and replace with --IgG2a--

Column 15, line 49, delete "IgG_{2a}" and replace with --IgG2a--

Column 15, line 50, delete "IgG₁" and replace with --IgG1--.

Column 15, line 46, delete "-7" and replace with --7--.

Column 16, line 2, delete "IgG₁" and replace with --IgG1--.

Column 16, line 3, delete "IgG_{2a}" and replace with --IgG2a--.

Column 16, line 18, delete "Mr" and replace with --M_r--.

Column 16, line 29, delete "are" and replace with --is--;
delete "IgG₁" and replace with --IgG1--.

Column 16, line 30, delete "IgG_{2a}" and replace with --IgG2a--

Column 16, line 44, delete "IgG_{2a}" and replace with --IgG2a--

Column 17, line 63 (claim 7), delete "P₃" and replace with --
P3--.

Column 18, line 57 (claim 17), delete "P₃" and replace with -
-P3--.

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INVENTOR(S): S.L. Morrison, Herzenberg, L.A., and Oi, V.T.

It is certified that error appears in the above-identified patent and that said Letters Patent are hereby corrected as shown below:

Column 19, line 33 (claim 27), delete "P₃" and replace with -
-P3--.

Column 20, line 50 (claim 41), delete "39" and replace with -
-37--.

Column 20, line 52 (claim 41), delete "are".

Column 20, line 53 (claim 41), delete "are".

Column 20, line 58 (claim 42), delete "are".

Column 20, line 59 (claim 42), delete "are".

Column 21, line 13, shift line beginning with "express" to
left margin.

Column 21, line 14, shift line beginning with "specifically"
to left margin.

Column 21, line 29 (claim 48), delete "arc" and replace with
--are--.

Column 22, line 43 (claim 59), delete "arc" and replace with
--are--.

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Docket No. BD/1 CIP FWCIV

Patent No: 5,807,715 Issued: September 15, 1998

Serial No: 08/266,154 Filed: June 27, 1994

RECEIPT IS HEREBY ACKNOWLEDGED OF THE
Request under 37 C.F.R. 1.322 and 1.323 for

Certificate of Correction in duplicate;

Exhibits A-D; Eight (8) Sheets of Corrections

in duplicate; Checks for \$100 and \$45

Dated June 25, 1999

Filed in connection with the above case.

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US005807715A

United States Patent [19][11] **Patent Number:** **5,807,715****Morrison et al.**[45] **Date of Patent:** **Sep. 15, 1998**

[54] **METHODS AND TRANSFORMED MAMMALIAN LYMPHOCYTE CELLS FOR PRODUCING FUNCTIONAL ANTIGEN-BINDING PROTEIN INCLUDING CHIMERIC IMMUNOGLOBULIN**

[75] **Inventors:** Sherie L. Morrison, Scarsdale, N.Y.;
Leonard A. Herzenberg, Stanford;
Vernon T. Oi, Menlo Park, both of Calif.

[73] **Assignee:** The Board of Trustees of The Leland Stanford Junior University, Stanford, Calif.

[21] **Appl. No.:** 266,154

[22] **Filed:** Jun. 27, 1994

Related U.S. Application Data

[63] Continuation of Ser. No. 893,610, Jun. 3, 1992, abandoned, which is a continuation of Ser. No. 675,106, Mar. 25, 1991, abandoned, which is a continuation of Ser. No. 441,189, Nov. 22, 1989, abandoned, which is a continuation of Ser. No. 90,669, Aug. 28, 1987, abandoned, which is a continuation-in-part of Ser. No. 644,473, Aug. 27, 1984, abandoned.

[51] **Int. Cl.^o** C12N 15/00; C12N 15/13;
C07K 16/00

[52] **U.S. Cl.** 435/69.6; 435/172.3; 435/326;
530/387.1; 530/387.3; 536/23.53

[58] **Field of Search** 435/69.6, 246.27,
435/320.1, 172.3, 326; 530/387.3, 387.1;
935/1.5; 536/23.53

[56] **References Cited**

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Seno et al 1983 *Nucleic Acid Research* 11(3):719-726.
Dolby et al 1980 *PNAS* 77(10) 6027-6031 Oct. 1980.
Siedman's Medical Dictionary 25th edition, p. 902, 1992.

Primary Examiner—Lila Feisec

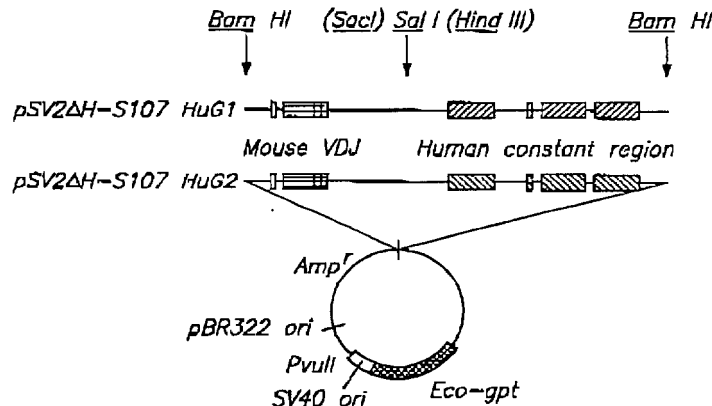
Assistant Examiner—Julie E. Reeves

Attorney, Agent, or Firm—Fish & Neave; Vicki S. Vcenker; Edward F. Mullenwey

[57] **ABSTRACT**

Methods for producing functional immunoglobulin are provided. The methods involve transfecting and expressing exogenous DNA coding for the heavy and light chains of immunoglobulin. In some embodiments, chimeric immunoglobulins are provided having variable regions from one species and constant regions from another species by linking DNA sequences encoding for the variable regions of the light and heavy chains from one species to the constant regions of the light and heavy chains respectively from a different species. Introduction of the resulting genes into mammalian host cells under conditions for expression provides for production of chimeric immunoglobulins having the specificity of the variable region derived from a first species and the physiological functions of the constant region from a different species.

62 Claims, 2 Drawing Sheets



U.S. Patent

Sep. 15, 1998

Sheet 1 of 2

5,807,715

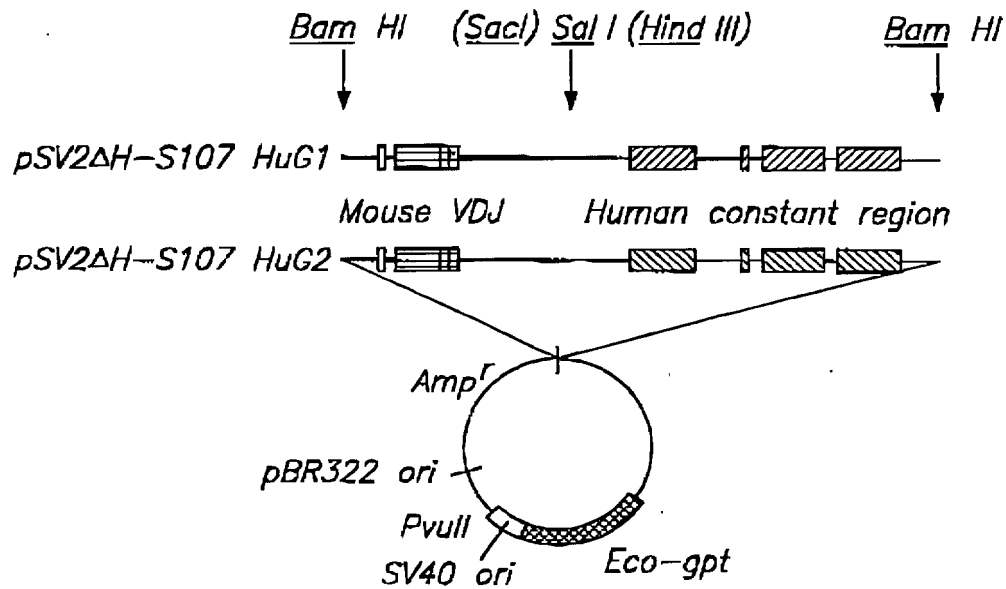


FIG. 1A

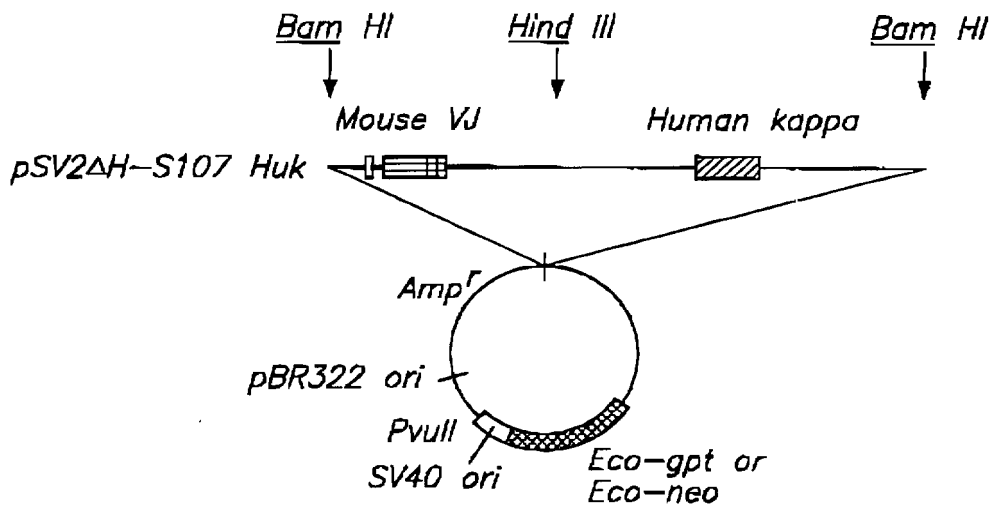


FIG. 1B

U.S. Patent

Sep. 15, 1998

Sheet 2 of 2

5,807,715

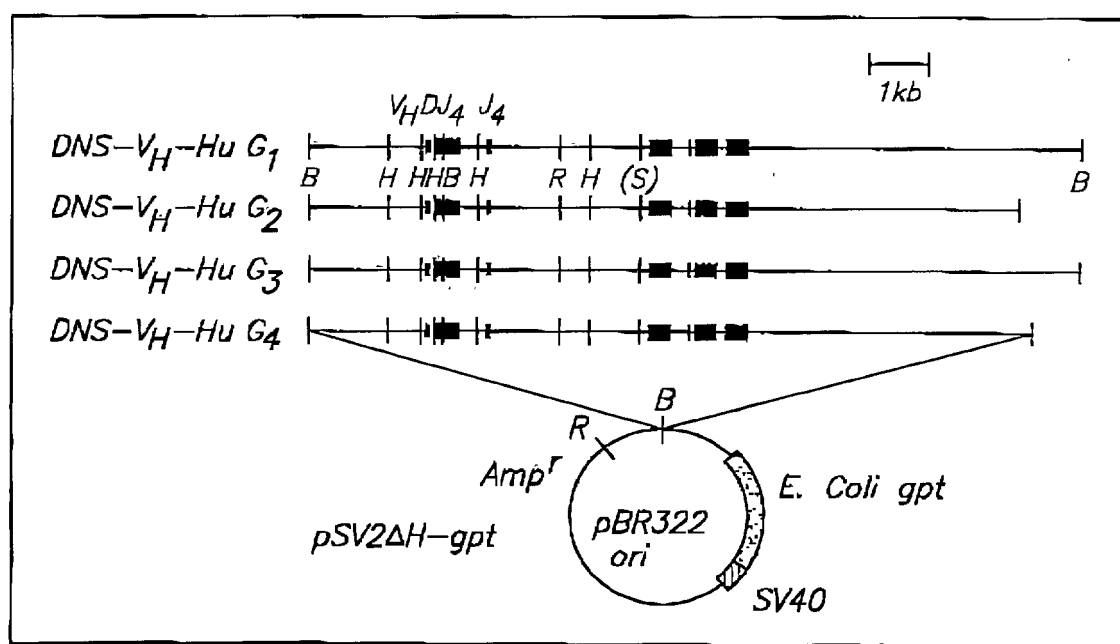


FIG. 2

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1

METHODS AND TRANSFORMED MAMMALIAN LYMPHOCYTE CELLS FOR PRODUCING FUNCTIONAL ANTIGEN- BINDING PROTEIN INCLUDING CHIMERIC IMMUNOGLOBULIN

This is a continuation of application Ser. No. 07/893,610, filed Jun. 3, 1992, now abandoned, which is a continuation of application Ser. No. 07/675,106, filed Mar. 25, 1991, now abandoned, which is a continuation of application Ser. No. 07/441,189, filed Nov. 22, 1989, now abandoned, which is a continuation of application Ser. No. 07/090,669, filed Aug. 28, 1987, now abandoned, which is a continuation-in-part of application Ser. No. 06/644,473, filed Aug. 27, 1984 now abandoned.

BACKGROUND OF THE INVENTION

1. Field of the Invention

Naturally occurring receptors, such as immunoglobulins, enzymes, and membrane proteins have seen an extraordinary expansion in commercial applications over the last decade. With the advent of monoclonal antibodies, the usefulness of immunoglobulins has been greatly expanded and in many situations has greatly extended prior uses employing polyclonal antibodies. However, in many applications, the use of monoclonal antibodies is severely restricted where the monoclonal antibodies are to be used in a physiological (in vivo) environment. Since, for the most part, monoclonal antibodies are produced in rodents, e.g., mice, the monoclonal antibodies are immunogenic to other species.

While the constant regions of immunoglobulins are not involved in ligand binding, the constant regions do have a number of specific functions, such as complement binding, immunogenicity, cell receptor binding, and the like. There will, therefore, be situations where it will be desirable to have constant regions which bind to cells or proteins from a particular species having binding regions for a particular ligand.

2. Relevant Literature

Kwan et al., *J. Exp. Med.* (1981) 153:1366-1370 and Clarke et al., *Nucl. Acids Res.* (1982) 10:7731-7749 describe V_H and V_L exons from the mouse phosphocholine-binding antibody-producing S107 myeloma cell line. Oi et al., *Proc. Natl. Acad. Sci. USA* (1983) 80:825-829, report that the mouse light chain gene is not expressed efficiently in a rat myeloma cell.

SUMMARY OF THE INVENTION

Chimeric multi-subunit receptors are provided, where each of the subunits is an expression product of a fused gene. Each fused gene comprises a DNA sequence from one host species encoding the region involved with ligand binding joined to a DNA sequence from a different source, either the same or a different host species, encoding a "constant" region providing a structural framework and biological properties. Introduction of the fused genes into an appropriate eukaryotic host cell under conditions for expression and processing provides for a functional assembled multi-subunit receptor product.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1A is a schematic diagram of the chimeric mouse-human heavy chain gene vector; and FIG. 1B is the chimeric light chain vector.

2

FIG. 2 is a schematic diagram of chimeric human IgG anti-DNS expression vectors.

DESCRIPTION OF THE SPECIFIC EMBODIMENTS

Novel methods and compositions are provided, for production of polypeptide products having specific binding affinities for a predetermined ligand and predetermined biological, particularly physiological, properties, each of which are not normally associated with the binding region peptide sequences. Particularly, multi-subunit chimeric receptors are provided which result from fused genes having the portion of the polypeptide involved with binding of a predetermined ligand having an amino acid sequence substantially the same (>90% conserved) as an amino acid sequence having the same function from one host, while the portion involved with providing structural stability, as well as other biological functions, being analogously derived from a different host. The resulting composition can be either an inter- or intraspecies chimera. At least two fused genes are involved, which genes are introduced into an appropriate eukaryotic host under conditions for expression and processing, whereby the fused genes are expressed and the resulting subunits bound together, resulting in an assembled chimeric receptor.

The receptors prepared in accordance with the subject invention will be multi-subunit, where the units are held together either by non-covalent binding or a combination of non-covalent and covalent binding, particularly disulfide linkages through cysteine, and having at least one binding site, usually at least two binding sites, and not more than about ten binding sites. Receptors of interest include both B-cell and T-cell receptors, more particularly, immunoglobulins, such as IgM, IgG, IgA, IgD and IgE, as well as the various subtypes of the individual groups. The light chain may be κ or λ . The heavy chains are referred to as μ , γ , α , δ , and ϵ .

In discussing the two regions of each subunit, the two regions will be referred to as "variable" and "constant" by analogy to immunoglobulins. The variable region is the region involved with ligand binding and, therefore, will vary in conformation and amino acid sequence depending upon the ligand. The region will usually be composed of a plurality of smaller regions (hypervariable or complementary determining regions), involving a region having as its primary function binding to the ligand (V) and a region associated with joining the V region to the constant region, the joining region (J). There may also be a hypervariable region joining the V and J regions, the diversity region (D). These regions are related to gene segments observed in the genes encoding immunoglobulin variable regions.

The constant region will not be associated with ligand binding and will be relatively limited in the variations in its conformation and amino acid sequence within any one species and within any one class, each class generally having from 1 to 4 subclasses. Each constant region is specific for a species. Within the classes there will be allotypes, individual polymorphisms within a class within a species.

The variable region of the immunoglobulins will be derived from a convenient mammalian source, which may be a rodent, e.g., mouse or rat, rabbit, or other vertebrate, mammalian or otherwise, capable of producing immunoglobulins. The constant region of the immunoglobulin, as well as the J chain for IgM and IgA (not the same as the J region of the heavy or light immunoglobulin chain), will be derived from a vertebrate source different from the source of the

5,807,715

3

variable region, particularly a mammalian source, more particularly primate or domestic animal, e.g., bovine, porcine, equine, canine, feline, or the like, and particularly, humans. The different source of the constant region can be either from a different species or from the same species as the mammalian source utilized to provide the variable region. Thus, the constant region of the receptor will normally be chosen in accordance with the purpose of the receptor. For example, where the receptor is to be introduced into the host, the constant portion will be selected so as to minimize the immune response of the host to the receptor and to optimize biological efficiency, such as complement fixation or physiological half-life (catabolism). Where the receptor is to bind to particular cell membrane surface receptors, the constant region will be chosen in accordance with the host of the receptor recognition site.

The fused gene derived from the two host sources will be prepared by joining the 5'-end of a sequence encoding the constant region in reading frame to the 3'-end of a sequence encoding the variable region. (In referring to 5' or 3' for a double strand, the direction of transcription is with 5' being upstream from 3'.) With immunoglobulins, two fused genes will be prepared, one for the light chain and one for the heavy chain. With T-cell receptors, the two fused genes will be for each of the two chains involved in the formation of the T-cell receptor. The DNA sequences employed for preparation of the fused gene may be derived from a variety of sources. These sources include genomic DNA, cDNA, synthetic DNA, and combinations thereof. The genomic DNA may or may not include naturally occurring introns.

The DNA obtained from natural sources, namely the genomic DNA or cDNA, may be obtained in a variety of ways. Host cells coding for the desired sequence may be isolated, the genomic DNA may be fragmented, conveniently by one or more restriction endonucleases, and the resulting fragments may be cloned and screened with a probe for the presence of the DNA sequence coding for the polypeptide sequence of interest. For the variable region, the rearranged germline heavy chain DNA will include V, D, and J regions, including the leader sequence, which may be subsequently removed as well as any introns. The rearranged germline light chain coding DNA will include the V and J regions including the leader sequence, as well as any introns which may be subsequently removed. The particular source of the exons defining the domains and the manner of splicing, where introns are present, is not germane to this invention. Once the cloned fragment has been identified which contains the desired DNA sequence, this fragment may be further manipulated to remove superfluous DNA, modify one or both termini, remove all or a portion of intervening sequences (introns), or the like.

In providing a fragment encoding the variable region, it will usually be desirable to include all or a portion of the intron downstream from the J region. Where the intron is retained, it will be necessary that there be functional splice acceptor and donor sequences at the intron termini. The gene sequence between the J (joining region) and the constant region of the fused gene may be primarily the intron sequence associated with (1) the constant region, (2) the J region, or (3) portions of each. The last may be a matter of convenience where there is a convenient restriction site in the introns from the two sources. In some instances, all or a portion of the intron may be modified by deletion, nucleotide substitution(s) or insertion, to enhance ease of manipulation, expression, or the like. When the variable region is chosen to be syngeneic with the host cells employed for expression, all or at least about 80% of the intron sequence can be

4

selected from the naturally occurring intron sequence associated with the J region. In some instances it will be necessary to provide adapters to join the intron or truncated intron to the constant region. By cleaving within the intron, the variable region will be separated from its natural constant region.

Alternatively, it may be desirable to have the fused gene free of the intron between the variable and constant regions. Thus, the 3' terminus will be at or in the joining region. Normally all or a portion of the J region will be associated with the host providing the variable region. By restriction enzyme analysis or sequencing of the J region, one can select for a particular site for the 3' terminus of the variable region.

Alternatively, one can use an exonuclease and by employing varying periods of digestion, one can provide for varying 3'-termini, which can then be used for linking to the constant region and selection made for a functional product in a variety of ways. For example, where joining of the variable region to the constant region results in a unique restriction site, the fused DNA fragments may be screened for the presence of the restriction site.

Alternatively, it may be found desirable to include an adapter or linker to join the variable region to the constant region, where the adapter or linker may have the same or substantially the same sequence, usually at least substantially the same sequence, of the DNA sequence of the two fragments adjacent the juncture. The adapter or linker will be selected so as to provide for the two sequences to be in common reading frame. Furthermore, by employing adapters, one could add an additional degree of variability in the binding affinity of the chimeric receptor, by providing for the expression of different amino acids in the J region.

The joining of the various fragments is performed in accordance with conventional techniques, employing blunt-ended or staggered-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and ligation with appropriate ligases.

For cDNA, the cDNA may be cloned and the resulting clone screened with an appropriate probe for cDNA coding for the desired variable or constant region. Once the desired clone has been isolated, the cDNA may be manipulated in substantially the same manner as the genomic DNA. However, with cDNA there will be no introns or intervening sequences. The cDNA is cleaved at or near the juncture of the variable region with the constant region so that the variable region is separated from the constant region and the desired region retained. Where a convenient restriction site exists, the cDNA may be digested to provide for a fragment having the appropriate terminus. The restriction site may provide a satisfactory site or be extended with an adapter. Alternatively, primer repair may be employed, where for the variable region a complementary sequence to the site of cleavage and successive nucleotides in the 3' direction of the complementary sequence is hybridized to the sense strand of the cDNA and the nonsense strand replicated beginning with the primer and removal of the single-stranded DNA of the sense strand 3' from the primer. The reverse is true for the constant region. Other techniques may also suggest themselves. Once the fragment has been obtained having the predetermined 3' or 5' terminus, as appropriate, it may then be employed for joining to the other region.

Finally, one or both of the regions may be synthesized and cloned for use in preparing the fused gene. For the most part,

5,807,715

5

the same or substantially the same constant region can be repetitively used, so that a library of constant regions may be established which can be selected for joining to variable regions. Thus, the constant regions would have an appropriate 5' terminus for joining directly or through an adapter to a variable region.

In order for expression of the fused gene, it will be necessary to have transcriptional and translational signals recognized by an appropriate eukaryotic host. For the most part, desirable eukaryotic hosts will be mammalian cells capable of culture in vitro, particularly leukocytes, more particularly myeloma cells, or other transformed or oncogenic lymphocyte, e.g., BBV transformed cells. Alternatively, non-mammalian cells may be employed, such as fungi, e.g., yeast, filamentous fungi, or the like.

The DNA sequence coding for the variable region may be obtained in association with the promoter region from genomic DNA. To the extent that the host cells recognize the transcriptional regulatory and translational initiation signals associated with the variable region, then the region 5' of the variable region coding sequence may be retained with the variable region coding sequence and employed for transcriptional and translational initiation regulation.

The contiguous non-coding region 5' to the variable region will normally include those sequences involved with initiation of transcription and translation, such as the TATA box, capping sequence, CAAT sequence, and the like. Usually the 5'-non-coding sequence will be at least 150 bp, more usually at least 200 bp, usually not exceeding about 2 kbp, more usually not exceeding about 1 kbp.

The non-coding region 3' to the constant region may be retained for its transcriptional termination regulatory sequences, such as termination and polyadenylation. Thus, by retaining the 3'-region naturally contiguous to the DNA sequence coding for the constant region, the transcriptional termination signals may be provided for the fused gene. Where the transcriptional termination signals are not satisfactorily functional in the expression host cell, then a 3' region functional in the host cell may be substituted. Conveniently, the non-coding 3' region may be obtained from a non-coding contiguous 3' region of a constant region from the expression host. The 3'-non-coding region may be joined to the constant region by any of the means described previously for manipulation and ligation of DNA fragments. This region could then be used as a building block in preparing the fused gene.

The fused gene for the most part may be depicted by the following formula:

TIR-(LS)_c-V_f-(VI)_e-(D)_b-(DI)_a-J-(I)_h-C-TTR

wherein:

TIR intends the transcriptional regulatory and translational initiation region and is generally of at least about 150 bp and not more than about 2 kbp, which may be in whole or in part the sequence naturally joined to the V coding region;

LS refers to a DNA sequence encoding a leader sequence and processing signal functional in the expression host for secretion and processing for removal of the sequence; this leader sequence can contain an intron, as is known in the art to occur;

c is 0 or 1;

V is a segment coding for the variable domain in reading frame with LS, when LS is present;

f is 0 or 1;

D is a segment coding for the diversity domain and is present for the heavy chain (b=1) and is absent for the light chain (b=0);

6

J is a segment coding for the joining region;

VI and DI are introns associated with the letter-indicated coding segments having functional donor and acceptor splicing sites;

a, b and c are the same or different and are 0 or 1, wherein when b is 0, c is 0; a, b, and c are all preferably 0;

I is an intron which may be naturally contiguous to the J segment or naturally contiguous to the C domain or a combination of fragments from both, or a fragment thereof, desirably including an enhancer sequence functional in said expression host, or I may be foreign in whole or in part to the J and C segments;

d is 0 or 1 (preferably 0);

C is the constant domain and may code for a μ , γ , δ , α or ϵ chain, preferably μ , γ , or α , usually including at least 80% of the constant region sequence, and may be the same as or a modified naturally occurring allotype or an altered constant region encoding an improved protein sequence; and

TTR is the transcriptional termination region providing for transcriptional termination and polyadenylation which may be naturally associated with C or may be joined to C, being functional in the expression host, usually being at least about 100 bp and may be 1 kbp or more.

Fused genes lacking, or containing modifications in, the hinge region or other immunoglobulin constant region domains can also be prepared, in like manner to the modifications described above, in which case the formula will be as shown above but with the hinge region of the constant chain being absent or modified.

The constructs for each of the different subunits may be joined together to form a single DNA segment or may be maintained as separate segments, by themselves or in conjunction with vectors.

The subunit constructs may be introduced into a cell by transformation in conjunction with a gene allowing for selection where the construct will become integrated into the host genome.

A large number of vectors are available or can be readily prepared which provide for expression in a host, either by maintenance as an extrachromosomal element or by integration into the host genome. For a mammalian host, a wide variety of vectors are known based on viral replication systems, such as Simian virus, bovine papilloma virus, adenovirus and the like. These vectors can be used as expression vectors where transcriptional and translational initiation and termination signals are present and one or more restriction sites are available for insertion of a structural gene. In addition, the vectors normally have one or more markers which allow for selection of host cells which contain the expression vector. The marker may provide for prototrophy to an auxotrophic host, biocide resistance, e.g., resistance to antibiotics, such as G418, or heavy metals, such as copper, or the like. If desired, expression vectors can be prepared by joining the various components, such as the replication system, markers, and transcriptional and translational regulatory initiation and termination signals in conjunction with the fused gene. Frequently, a vector will include a prokaryotic replication system, which allows for cloning, manipulation, purification, and expansion of the desired DNA sequence.

A wide variety of transcriptional and translational regulatory sequences may be employed, depending upon the nature of the host. The transcriptional and translational regulatory signals may be derived from viral sources, such as adenovirus, bovine papilloma virus, Simian virus, or the like, where the regulatory signals are associated with a particular gene which has a high level of expression.

5,807,715

7

Alternatively, promoters from mammalian expression products, such as actin, collagen, myosin, etc., may be employed. Transcriptional initiation regulatory signals may be selected which allow for repression or activation, so that expression of the fused genes can be modulated. Of interest are regulatory signals which are temperature-sensitive so that by varying the temperature, expression can be repressed or initiated, or are subject to chemical regulation, e.g., metabolite.

Once the vector DNA sequence containing the fused gene has been prepared for expression, the DNA construct may be introduced into an appropriate host. Various techniques may be employed, such as protoplast fusion, calcium phosphate-precipitation, or other conventional technique. After the fusion, the cells are grown in a selective medium or are phenotypically selected leaving only cells transformed with the DNA construct. Expression of the fused gene results in assembly to form the receptor. To date, expression has been accomplished in lymphocytes.

The host cells will for the most part be immortalized cells, particularly myeloma or lymphoma cells. These cells may be grown in an appropriate nutrient medium in culture flasks or injected into a syngeneic host, e.g., mouse or rat, or immunodeficient host or host site, e.g., nude mouse or hamster pouch. Particularly, the cells may be introduced into the abdominal cavity for production of ascites fluid and harvesting of the chimeric receptor. Alternatively, the cells may be injected subcutaneously and the antibodies harvested from the blood of the host. The cells may be used in the same manner as hybridoma cells. See Diamond et al., *N. Eng. J. Med.* (1981) 3034:1344 and Kennatt, McKearn and Bechtol (eds.), *Monoclonal Antibodies: Hybridomas—A New Dimension in Biologic Analysis*, Plenum, 1980, which are incorporated herein by reference.

Where a leader is present with a processing signal for secretion and selective cleavage of the leader (signal) sequence, the resulting assembled receptor will be secreted into the nutrient medium of the transformed cells and may be harvested. Where secretion does not occur, after sufficient time for the receptor to be expressed in reasonable amounts, the cells may be killed, lysed, and the receptors isolated and purified. Where transcriptional initiation can be modulated, the cells may be grown to high density under non-permissive conditions, followed by growth under permissive conditions where the receptor is expressed.

The receptors may be naturally glycosylated, unnaturally glycosylated or be free of glycosyl groups, depending on the host, conditions of cellular growth and subsequent treatment. Where a mammalian host cell is employed for expression, usually natural glycosylation will occur. Glycosylation can be prevented by an appropriate inhibitor, e.g., tunicamycin. Alternatively, glycosyl groups may be removed by hydrolysis, e.g., enzymatic hydrolysis using hydrolases. In expression hosts other than mammalian cells, unglycosylated or unnatural glycosylated receptors may be obtained.

The receptor may be isolated and purified in accordance with conventional conditions, such as extraction, precipitation, chromatography, affinity chromatography, electrophoresis, or the like. By employing antibodies specific for the constant region(s), affinity chromatography will allow for concentration and purification of the chimeric receptor.

The chimeric receptors can be used in the same manner as other receptors for binding to specific ligands in diagnostic assays, affinity chromatography or the like. In addition, because a chimeric receptor of substantially reduced immu-

8

nogenicity can be produced, the chimeric receptors can find use in therapy, for passive immunization for in vivo imaging, for specific treatment of diseased cells, or the like. For in vivo imaging, the chimeric antibody will normally be conjugated to a radionuclide, e.g., technetium, rhenium, or the like. For biocidal activity, the antibody may be joined to the A-portion of toxins, liposomes containing biocidal reagents, radionuclides, or other biocidal agent. Alternatively, the antibodies can be used in combination with the host immune system, e.g., complement, due to the presence of the native constant region. In vitro, the subject chimeric antibodies can be used in conjunction with complement to remove particular cells from a mixture of cells, where the target cells have a ligand complementary to the binding site of the chimeric antibody.

The following examples are offered by way of illustration and not by way of limitation.

EXAMPLE 1

Materials and Methods

Chimeric Genes

The cloned S107 variable region (heavy) (VH) and S107 Vκ variable region (light, kappa) genes were obtained from Dr. Matthew Scharff (Dept. of Cell Biology, Albert Einstein College of Medicine, Bronx, N.Y. 10641). The S107 VH gene was spliced to human IgG1 and IgG2 constant region genes using SalI linkers as shown in FIG. 1A. Both constructs were inserted into the vector pSV2AH-gpt (Oi et al., *Proc. Natl. Acad. Sci. USA* (1983) 80:825-829; Mulligan and Berg, *Science* (1980) 209:1422-1427). The S107 Vκ gene was spliced to the human κ gene at a unique HindIII site located in the large intron between the Jκ and Cκ exons as shown in FIG. 1B. This chimeric light chain gene construct was inserted into both pSV2AH-gpt and pSV2-neo plasmid vectors (Mulligan and Berg, *Proc. Natl. Acad. Sci. USA* (1981) 78:2072-2076) and pSV184AH-neo plasmid vectors (Oi and Morrison, *Biotechniques* (1986) 4:214-221).

Transfection

Protoplast fusion and calcium-phosphate (CaPO₄) precipitation techniques (Oi et al., (1983) supra; Sandri-Goldin et al., *Mol. Cell. Biol.* (1981) 1:743-752; Chu and Sharp, *Gene* (1980) 13:197-202) were used to transfect these chimeric immunoglobulin genes into the J558L myeloma cell line (a lambda (λ) light chain producing mouse myeloma cell line) and the non-immunoglobulin-producing An derivative of the P3 myeloma cell line. Mycophenolic acid (Gibco Laboratories, Santa Clara, Calif. 95050) was used for selection of cells transfected with pSV2AH-gpt vectors as described previously (Oi et al., (1983) supra; Ochi et al., *Proc. Natl. Acad. Sci. USA* (1983) 80:6351-6355). G418 (Gibco Laboratories) at 1.0 mg/ml was used for selection of cells transfected with pSV2-neo vectors (Mulligan and Berg, (1980) supra).

When both light and heavy chimeric genes were transfected into the J558L cell line using protoplast fusion techniques, light and heavy chimeric immunoglobulin genes were transfected sequentially using G418 selection for the chimeric light chain gene vector and mycophenolic acid for the chimeric heavy chain gene vector. The protoplast fusion transfection procedure used was as described previously (Oi et al., (1983) supra).

Transfection using the calcium phosphate precipitation procedure was done by mixing 40 μg of both chimeric light and chimeric heavy chain pSV2AH-gpt vectors and transfecting a total of 80 μg of plasmid DNA into 5×10⁶ cells. Mycophenolic acid was used to select for transformed cell lines as described previously (Oi et al., (1983) supra).

5,807,715

9

Antigen-binding

Phosphocholine (PC) binding of antibody secreted into the culture supernates of transfected cell lines was analyzed using a solid-phase radioimmunoassay described previously (Oi and Herzenberg, *Mol. Immunol.* (1979) 16:1005-1017). PC-binding antibodies in biosynthetically-labeled culture supernates and cell lysates of transfected cell lines also were analyzed by binding the biosynthetically-labeled antibody to PC-coupled to Sepharose 4B (Pharmacia Fine Chemicals, Piscataway, N.J.) and then eluting the bound antibody with PC-hapten. The bound and eluted antibody was examined by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Biosynthetic-labeling procedures were done as described previously (Oi et al., *J. Exp. Med.* (1980) 151:1260-1274). Idiotype Analysis

Three hybridoma anti-idiotypic antibodies, obtained from Dr. Matthew Scharff (Dept. of Cell Biology, Albert Einstein College of Medicine, Bronx, N.Y.), were used to analyze the variable heavy-variable light (VH-VL) domain structure of the chimeric human anti-PC antibodies. These antibodies, recognizing three independent idiotopes, were used to immunoprecipitate biosynthetically-labeled material eluted with PC from the PC-Sepharose 4B matrix. Immunoprecipitates were analyzed by SDS-PAGE.

Immunoglobulin Chain Composition

Monoclonal anti-human IgG and anti-human κ antibodies (Becton-Dickinson Monoclonal Center, Mt. View, Calif.) were used to immunoprecipitate biosynthetically-labeled chimeric human anti-PC antibodies for analyses using two-dimensional non-equilibrium pH gradient polyacrylamide gel electrophoresis (NEPHGE) (Oi and Herzenberg, (1979) supra). PC-coupled to Sepharose 4B also was used for immunoprecipitations.

Immunoglobulin Heavy Chain Glycosylation

Tunicamycin (Calbiochem-Behring, San Diego, Calif.) was used to inhibit asparagine-linked glycosylation of biosynthetically-labeled antibody from mouse cell lines producing mouse:human chimeric immunoglobulins (Oi et al., (1980) supra). PC-binding antibody from tunicamycin-treated cells was analyzed by SDS-PAGE. Procedures used for tunicamycin treatment were as described previously (Oi et al., (1980) supra).

Chimeric Mouse:Human Antibody Production in Mice

Transformed J558L cells producing chimeric mouse:human antibody were injected subcutaneously into BALB/c mice (10^6 cells/mouse). Sera from tumor-bearing mice were analyzed for human anti-PC antibody by a solid-phase radioimmunoassay described previously (Oi and Herzenberg, (1979) supra) and by immunoelectrophoresis using a polyclonal anti-human antiserum.

Results

Expression of chimeric mouse V:human C region genes in transfected mouse myeloma cells J558L and the non-immunoglobulin-producing P3 myeloma cell lines was obtained. When both light chain and heavy chain chimeric genes were transfected into the same cell, tetrameric (H2L2) antigen-binding antibodies were obtained. Autoradiograms of two-dimensional NEPHGE analyses of the chain composition of biosynthesized and secreted antibody molecules bound and eluted from phosphocholine-Sepharose showed the formation of mixed molecules, including the endogenously produced J558L λ light chain. Each polypeptide chain had the expected charge and relative molecular weight. Identical two-dimensional gel analyses results were obtained with immunoprecipitates with monoclonal anti-human κ and IgG antibodies. Similar results were obtained

10

from immunoprecipitates of human IgG(κ) antibodies produced by transfected P3 cell lines. Since the non-immunoglobulin-producing parental P3 cell line does not produce endogenous immunoglobulin polypeptide chains, as expected only the chimeric mouse:human heavy and light chains are seen on the autoradiograms.

Phosphocholine-binding by the chimeric antibody produced in the J558L cell line was the result from specific association of the chimeric immunoglobulin light and heavy polypeptide chains, i.e., the VH and V κ domains of the S107 myeloma protein connected to human constant region polypeptides. This was determined by measuring PC-binding by immunoglobulins produced by J558L cells transfected with the mouse: human chimeric heavy chain gene. PC-binding was never observed from antibody secreted by transfected J558L cells expressing only the chimeric heavy chain and the endogenous J558L λ light chain (data not shown). Chimeric antibodies produced in the transfected non-immunoglobulin-producing P3 cell line also were shown to bind PC-Sepharose. In view of the low binding affinity of the parental mouse S107 antibody, analyses of appropriate polypeptide folding of mouse VH and V κ domains in the novel environment of human constant regions polypeptide chains were done by determining the presence of idiotypes known to occur on the parental S107 PC-binding antibody molecule. Three monoclonal anti-idiotypic antibodies, each recognizing a distinct epitope on the light and heavy variable region domains and an epitope defined by the presence of both light and heavy variable region domains, were found to react with the mouse:human chimeric anti-PC antibodies. This strongly supports the fact that the mouse S107 antigen-binding domains have folded into their intended structures.

Glycosylation of the mouse:human chimeric antibodies in mouse myeloma cells was analyzed by measuring the relative molecular weight (Mr) of antibodies biosynthesized in the presence and absence of tunicamycin, a known antibiotic inhibitor of asparagine-linked glycosylation. Autoradiograms of SDS-PAGE analysis of the chimeric heavy and light chains produced in mouse myeloma cells in the presence or absence of tunicamycin showed the lower relative Mr of the heavy chain synthesized in the presence of tunicamycin as expected if a single N-linked carbohydrate was absent from the polypeptide chain. From these data it is concluded that the mouse myeloma cell appropriately glycosylates the human heavy chain.

When transfected J558L cells producing the human IgG2 (κ) chimeric anti-PC antibody were grown as a subcutaneous tumor in BALB/c mice, analysis of the sera of these mice showed significant human IgG2(κ) anti-PC binding antibody production by radioimmunoassay. Polyclonal anti-human antiserum demonstrated the presence of significant quantities of human immunoglobulin in the sera. Based on comparison with prior experience with mouse hybridoma antibody production in mice, the amount of immunoglobulin visualized by immunoelectrophoresis analysis of mice bearing tumors of the transfected J558L cell line was similar to the lower levels of production seen with other mouse hybridoma tumor cell lines.

Analysis showed that fewer than about 10% of the transfected cell lines produced both chimeric heavy and light chain polypeptides. Among transformants generated by protoplast fusion, both gpt and neo biochemical markers were expressed at expected frequencies. However, chimeric light chain expression was infrequent. In co-transfection experiments using the CaPO₄ precipitation protocol, the same phenomenon was observed.

5,807,715

11

Based on prior experience with co-expression of gene products in transformed cell lines, it appears that the appropriate transcriptional or translational controlling elements are absent in either the chimeric light chain gene construct or in the mouse myeloma cell lines used. The mouse V_k gene promoter is coupled to the presumed human intronic DNA sequences that are homologous to the known mouse intronic controlling element (ICE) or immunoglobulin "enhancer" element (Morrison and Oi, *Ann. Rev. Immunol.* (1984) 7:239-256). The chimeric mouse: human heavy chain gene is not constructed in this manner and, in contrast, is expressed efficiently. The mouse heavy chain intronic controlling element (ICE) sequences are included and human sequences excluded in this construct. The basis for the low level of expression of the light chain is subject to speculation.

It is evident from the above results that chimeric receptors, as illustrated by immunoglobulins, can be produced where the variable regions may be obtained from one host source and the constant regions obtained from another host source. Where the immunoglobulins are to be used in vivo, this can provide for numerous advantages, such as reduced immunogenicity, a lower catabolism, and the ability to fulfill biological effector functions associated with the constant regions. Furthermore, now that it is shown that chimeric receptors can be produced, there is the opportunity to prepare chimeric receptors with modification of V, J, and D regions so as to modify binding specificity.

EXAMPLE 2

A family of recombinant anti-DNS antibodies was prepared by cloning the mouse V_H gene expressed in the DNS1 (27-44) hybridoma cell line (Dangl et al., *Cytometry* (1982) 2:395) and joining this gene to the already cloned human IgG_1 , IgG_2 , IgG_3 , IgG_4 , rabbit IgG and mouse IgG_3 heavy chain constant region genes. These recombinant genes were inserted into the eukaryotic expression vector, pSV2(delta) H-gpt (Oi et al., *BioTechniques* (1987) 4:214). Each recombinant vector was transfected into a heavy chain loss mutant of the DNS1 hybridoma cell line, 27-44-ASC13, or co-transfected with a DNS- V_k expression vector into the Ig^- cell line SP2.0 to generate stable anti-DNS transfectoma cell lines (Morrison and Oi, *Ann. Rev. Immunol.* (1984) 2:239). The expressed V_H and V_k genes from the DNS1 hybridoma (Oi et al., *Nature* (1984) 307:136; Reidler et al., *J. Mol. Biol.* (1982) 158:739) were cloned from phage lambda libraries (Dangl, thesis, Stanford University, Stanford, Calif.) and used to construct the vectors shown in FIG. 2. Transfection by protoplast fusion and selection and screening of transfectomas has been described (Oi et al., *BioTechniques*, supra; Dangl, thesis, Stanford University, Stanford, Calif.). Anti-DNS antibodies were purified from culture supernate by affinity chromatography using a dansyl isomer 2-dimethylaminonaphthyl-5-sulfonamide-3-lysine as absorbant (coupled to AII-Sepharose-4B) and eluant. The affinity of the DNS1 combining site for DNS is 17 nM; the binding affinity of this isomer is lower by a factor of 10^3 , making it suitable for use in affinity purification. Removal of bound hapten by dialysis was monitored by fluorescence emission spectroscopy. Antibodies were pure, >95% and free of aggregates as determined by size exclusion chromatography (Dangl, thesis, Stanford University, Stanford, Calif.). The chimeric mouse-human immunoglobulin heavy chain vectors are depicted in FIG. 2. The rabbit IgG and mouse IgG_3 heavy chain vectors were similarly constructed.

The nature of the DNS combining site of the genetically engineered antibodies was monitored by measuring the

12

fluorescence emission spectra of bound DNS-lysine. This hapten is a sensitive indicator of the dynamic polarity of its microenvironment. Independently derived mouse IgG_1 anti-DNS antibodies with different combining sites were previously shown to generate different emission spectra (Reidler et al., *J. Mol. Biol.* (1982) 158:739). The absorption and emission spectra of DNS-lysine bound to each genetically engineered antibody was identical to the DNS1 combining site of the parental hybridoma (corrected fluorescence emission spectra of each affinity purified chimeric antibody were measured using a SIM model 8000 fluorescence spectrophotometer with 340 nm excitation, essentially as described in Reidler, et al., *J. Mol. Biol.* (1982) 158:739). This indicates that each chimeric antibody has a properly folded V_H domain, despite the heterologous junction between the mouse V_H and human or rabbit C_H domains. Stable transfectoma cell lines produced immunoglobulins with heavy chains of appropriate size and charge as determined by two-dimensional sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Immunoglobulins produced by each transfectoma cell line were analyzed by biosynthetic labelling, immunoprecipitation and one- or two-dimensional SDS-PAGE essentially as described by P. P. Jones in *Selected Methods in Cellular Immunology*, B. B. Mishell, S. M. Shilgi, Eds. (W. H. Freeman, San Francisco, 1980), pp. 238. Rabbit anti-human IgG anti-sera was purchased from Cappel. The recombinant heavy chains are glycosylated, a post-translation modification known to affect biosynthetic labeling in the presence or absence of tunicamycin, an antibiotic known to inhibit asparagine-linked glycosylation (Hickman and Kornfeld *J. Immunol.* (1978) 121:990; Nose and Wiggall *Proc. Natl. Acad. Sci.* (1983) 80:6632; Leatherbarrow et al. *Molec. Immunol.* (1985) 22:407).

This Example demonstrates the ability to prepare inter-species chimeric antibodies from additional species using the previously described techniques as well as the ability to produce intra-species chimerics.

EXAMPLE 3

A series of intra-species hybrid mouse IgG_1 - IgG_{2a} immunoglobulins all with identical light chains and variable regions were produced. Hybrid heavy chain constant region gene segments were generated by genetic recombination in *E. coli* between plasmids carrying mouse $\gamma 1$ and $\gamma 2a$ gene segments. Crossovers occurred throughout these segments although the frequency was highest in regions of high nucleotide sequence homology. Eleven hybrid proteins were produced by transfection of a variant hybridoma cell line. Immunoglobulins produced by the transfected cell lines were assembled into H_2L_2 tetramers and properly glycosylated in addition to having identical antigen combining sites specific for the fluorescent hapten e-dansyl-L-lysine. Protein A binding, used as a probe of Fc structure in these variant antibodies was consistent with the identity of protein A contact residues within the Fc. Novel receptors with new and improved functions were created, demonstrating that improvements over naturally occurring receptors can be made.

Materials and Methods

Generation of hybrid genes

Hybrid C_H gene segments coding for polypeptides with an IgG_1 amino-terminus and an IgG_{2a} carboxy-terminus or an IgG_{2a} amino-terminus and IgG_1 carboxy-terminus were generated by adaptation of the system described by Schneider et al., *Proc. Natl. Acad. Sci.* (1981)

5,807,715

13

78:2169-2173. Plasmid pHGX1 was constructed by digesting pBN2 (Nichols and Yanofsky, *Proc. Natl. Acad. Sci.* (1979) 76:5244-5248) with HindIII and PvuII, filling in the overhanging HindIII ends with T₄ DNA polymerase, ligating XbaI linkers to the flush ends, and recircularizing the plasmid. Plasmids pHGX1C_{γ2a}A and pHGX1C_{γ2a}B were generated by digesting pγ2a.9 with EcoRI and HindIII, filling in the overhanging ends, ligating XbaI linkers to the blunt ends, and inserting the C_{γ2a}-containing fragment into XbaI-digested pHGX1. Plasmids pHGX1C_{γ2a}A and pHGX1C_{γ2a}B differ only in the orientation of the inserted fragment. Plasmid pHGX2 was constructed by digesting pWS1 (Schneider et al., *Proc. Natl. Acad. Sci.* (1981) 78:2169-2173) with HpaI and SalI, filling in the overhanging SalI end, ligating XbaI linkers to the flush ends, and recircularizing the plasmid. Plasmids pHGX2C_{γ1}A and pHGX2C_{γ1}B were generated by digesting P_{γ1} with KpnI, and ligating the C_{γ2a}-containing fragment to XbaI-digested pHGX2. Plasmids pHGX2C_{γ1}A and pHGX2C_{γ1}B differ only in the orientation of the inserted fragment.

E. coli strain W3110 trpR AtrpEA2 tnaA2 tna-19 was transformed with both pHGX1C_{γ2a}A and pHGX1C_{γ1}A or pHGX1C_{γ2a}B and pHGX1C_{γ1}B to chloramphenicol resistance (Cm^r) and ampicillin resistance (Amp^r). Single colonies were transferred to L broth containing chloramphenicol (20 mg/l) and ampicillin (100 mg/l) and grown overnight. Cells were collected by centrifugation, washed with Vogel-Bonner minimal medium (Vogel and Bonner, *J. Biol. Chem.* (1956) 218:97-106), and plated on minimal plates supplemented with glucose (0.4%), acid-hydrolyzed casein (0.5%), indole (10 mg/l) and chloramphenicol. Individual colonies were transferred to identical liquid medium and grown overnight. Plasmid DNA was extracted from these cultures, and the monomeric double crossover plasmids isolated by size fractionation using agarose gel electrophoresis. DNA from monomeric plasmid fractions was used to transform *E. coli* W3110 trpR AtrpEA2 tnaA2 tna-19 to Trp⁺ and Cm^r. Transformants were screened for ampicillin sensitivity (Amp^r) by replica plating.

The crossover site generating each hybrid was located by restriction enzyme analysis. Precise crossover junctions were identified by DNA sequencing by the method of Maxam and Gilbert *Methods Enzymol.* (1980) 65:499-560 or Sanger and Coulson, *Proc. Natl. Acad. Sci.* (1977) 74:5463-5467 using M13mp9 (Messing and Vieira, *Gene* (1982) 19:269-276) to generate single-stranded DNA template.

An additional variant heavy chain gene, one lacking the hinge region exon, also was constructed. The XbaI ends of fragments containing C_H gene segments were filled in and converted to SalI ends with linkers. These fragments were ligated into the SalI site of pMLSVgpt DNS-V_HAγ_{2a} heavy chain constant region with a hinge exon deletion was constructed by removing a SmaI-SmaI fragment from an expression vector carrying the γ_{2a} C_H coding region. This plasmid is designated pMLSVgpt DNS-V_HC_{γ2a}Δ hinge.

Transfection of Hybrid Heavy Chain Genes

Plasmids designed to express anti-DNS hybrid heavy chain genes were constructed as described above. The pMLSVgpt DNA-V_H contains the *E. coli* xanthine-guanine phosphoribosyltransferase gene (gpt) which is used to biochemically-select transfected mammalian cells (Mulligan and Berg, *Proc. Natl. Acad. Sci.* (1981) 78:2072-2076). The heavy chain variable region (V_H) gene segment from the anti-DNS hybridoma 27-44 (Dangi et al., *Cytometry* (1982) 2:395-401) is located upstream of the C_H gene insertion site. Hence ligation of each recombinant

14

heavy chain constant region gene segment into pMLSVgpt DNA-V_H in the proper orientation generates a gene encoding the corresponding variant anti-DNS heavy chain polypeptide. Hybrid anti-DNS antibody-producing cell lines were generated by transfecting these recombinant vectors into a heavy-chain-loss variant of the 27-44 hybridoma cell line, designated 27-44 A5C13 (which was provided by Dr. D. Parks of Stanford University). Other hybridoma cell lines lacking production of the heavy chain could also be used.

Expression vectors were transfected into 27-44 A5C13 using the following protoplast fusion procedure. *E. coli* strain HB101 carrying the appropriate plasmid was grown in L broth to an OD₆₀₀ of 0.6-0.7. Plasmid copy number was amplified by addition of chloramphenicol (170 mg/l) and incubating cultures overnight. Cells from 25 ml of culture were harvested by centrifugation and gently resuspended in 1.25 ml of ice cold 20% sucrose, 50 mM Tris-HCl pH 8.0. Freshly prepared lysozyme solution (0.25 ml at 5 mg/ml in 0.25M Tris-HCl pH 8.0) was added and the suspension incubated for 6 minutes on ice. A 0.5 ml aliquot of 0.25 mM EDTA pH 8.0 was added and the mixture was incubated for 5 minutes on ice. Following addition of 0.5 ml of 50 mM Tris-HCl pH 8.0, the cells were incubated for 10 minutes at 37° C. The bacteria were then diluted with 10 ml of RPMI 1640 medium supplemented to 10% sucrose and 10 mM MgCl₂ and warmed to 37° C. After incubation for 10 minutes at room temperature, the protoplasts were used for fusion.

The 27-44 A5C13 cell line was grown to a density of 0.3 to 1×10⁶ cells/ml in RPMI 1640 medium containing 1.0 mM sodium pyruvate, 2.0 mM L-glutamine, 50 mM β-mercaptoethanol, 10% fetal calf serum (FCS), and 1% NuSerum™ (Collaborative Research, Lexington, Mass.). Cells (2×10⁷) were harvested by centrifugation and resuspended in 1 ml of RPMI 1640 medium with supplements. After addition of 3 ml of protoplast suspension, the cells were pelleted by centrifugation. The pellet was resuspended by slowly adding 1 ml of 45% polyethylene glycol (PEG) in RPMI 1640 medium without supplements (warmed to 37° C.) while mixing gently for 1 to 2 minutes. Nine milliliters of 37° C. RPMI medium without supplements were added slowly to dilute the PEG solution. The suspension was centrifuged and the pellet resuspended in 12 ml of RPMI 1640 medium. Cells were then dispensed into a 24-well plate in 0.5 ml aliquots. The following day, 1 ml of RPMI 1640 medium was added to each well. Two days after fusion, transfected cell lines were selected in medium containing 1 mg/l mycophenolic acid, 15 mg/l hypoxanthine, and 200 mg/l xanthine. Cultures were fed with 1 ml of selection medium as needed.

Protein Characterization

Antibodies from transfected cell lines were biosynthetically-labelled with [³⁵S]-methionine in the absence and presence of tunicamycin. Antibodies were immunoprecipitated with rabbit anti-mouse immunoglobulin antisera and analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE), two-dimensional non-equilibrium pH gradient gel electrophoresis, and two-dimensional non-reducing-reducing polyacrylamide gel electrophoresis (Goding, *Handbook of Experimental Immunology*, Vol. 1. Blackwell Scientific Publications, Oxford (1986) 20:1-20:33). Fluorescence emission spectra of hybrid anti-DNS antibodies bound to ε-dansyl-L-lysine were measured using 340 nm excitation on a SLM model 8000 fluorescence spectrophotometer.

Production and Purification of Genetically Engineered Antibodies

The protein A binding of each of the eleven variant antibodies was assessed and used as a probe of Fc structural integrity. Table 1 summarizes the results of this analysis.

[illegible]

5,807,715

17

18

TABLE 1-continued

Amino acid residues at positions of protein A contact and protein A binding of hybrid and hinge dele- ted antibodies													
Antibody	Contact Residues										Protein A		
	252	253	254	308	309	310	311	312	433	434	435	436	Binding
$\gamma_1\gamma_{2a}$ ⁻⁵	-	-	-	-	-	-	-	-	-	-	-	-	+
IgG _{2b} Δhinge	-	-	-	-	-	-	-	-	-	-	-	-	+
$\gamma_1\gamma_{2a}$ ⁻⁸ (IgG1)	Thr	-	Thr	-	Met	-	-	-	-	-	-	-	-
$\gamma_1\gamma_{2a}$ ⁻⁶	Thr	-	Thr	-	Met	-	-	-	-	-	-	-	-
$\gamma_1\gamma_{2a}$ ⁻⁷	Thr	-	Thr	-	Met	-	-	-	-	-	-	-	-
$\gamma_1\gamma_1$ ⁻¹	Thr	-	Thr	-	Met	-	-	-	-	-	-	-	-
$\gamma_1\gamma_1$ ⁻²	Thr	-	Thr	-	Met	-	-	-	-	-	-	-	-
$\gamma_1\gamma_{2a}$ ⁻⁴	Thr	-	Thr	-	-	-	-	-	-	-	-	-	-
$\gamma_1\gamma_{2a}$ ⁻³	Thr	-	Thr	-	-	-	-	-	-	-	-	-	-

Contact residues in $\gamma_1\gamma_2$ are given. EU numbering is used.

All publications and patent applications mentioned in this specification are indicative of the level of skill of those skilled in the art to which this invention pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

The invention now being fully described, it will be apparent to one of ordinary skill in the art that many changes and modifications can be made thereto without departing from the spirit or scope of the appended claims.

What is claimed is:

1. A method for producing a functional immunoglobulin comprising a heavy chain and a light chain, which comprises the steps of:

- transfecting a transformed mammalian lymphocytic cell with a first DNA molecule coding for a first chain of the immunoglobulin;
- transfecting the cell with a second DNA molecule, said second DNA molecule coding for a second chain of the immunoglobulin, said second chain being a chain other than the first chain and said first and second chains being either the heavy chain or the light chain; and
- maintaining the cell in a nutrient medium, so that the cell expresses the first and second DNA molecules and the resultant chains are intracellularly assembled together to form the immunoglobulin which is then secreted in a form capable of specifically binding to antigen

wherein prior to step (a) the cell does not express a functional immunoglobulin capable of specifically binding antigen.

2. A method as recited in claim 1 wherein the cell is transfected via protoplast fusion.

3. A method as recited in claim 1 wherein the cell is transfected via calcium phosphate precipitation.

4. A method as recited in claim 1 wherein the cell is a myeloma cell.

5. A method as recited in claim 4 wherein the cell is a murine myeloma cell.

6. A method as recited in claim 1 wherein the cell does not endogenously produce any immunoglobulin chains.

7. A method as recited in claim 6 wherein the cell is a murine P₃ cell.

8. A method as recited in claim 1 wherein prior to step (a) the cell endogenously produces an immunoglobulin light chain or an immunoglobulin heavy chain, but not both.

9. A method as recited in claim 8 wherein the cell is a murine J558L cell.

10. A method as recited in claim 1 wherein the immunoglobulin comprises the variable region found in a first mammalian species and comprises the constant region found in a second mammalian species, said second mammalian species being other than the first mammalian species.

11. A method for producing a functional immunoglobulin comprising a heavy chain and a light chain, which comprises the steps of:

- transfecting a transformed mammalian lymphocytic cell with a plasmid comprising a first DNA molecule coding for a first chain of the immunoglobulin and a second DNA molecule coding for a second chain of the immunoglobulin, said second chain being a chain other than the first chain and said first and second chains being either the heavy chain or the light chain; and
- maintaining the cell in a nutrient medium so that the cell expresses said first DNA molecule and said second DNA molecule and the resultant chains are intracellularly assembled together to form the immunoglobulin which is then secreted in a form capable of specifically binding to antigen

wherein prior to step (a) the cell does not express a functional immunoglobulin capable of specifically binding antigen.

12. A method as recited in claim 11 wherein the cell is transfected via protoplast fusion.

13. A method as recited in claim 11 wherein the cell is transfected via calcium phosphate precipitation.

14. A method as recited in claim 11 wherein the cell is a myeloma cell.

15. A method as recited in claim 14 wherein the cell is a murine myeloma cell.

16. A method as recited in claim 11 wherein the cell does not endogenously produce any immunoglobulin chains.

17. A method as recited in claim 16 wherein the cell is a murine P₃ cell.

18. A method as recited in claim 11 wherein prior to step (a) the cell endogenously produces an immunoglobulin light chain or an immunoglobulin heavy chain, which endogenously-produced heavy chain is not secreted in a form capable of specifically binding to antigen, but not both.

19. A method as recited in claim 18 wherein the cell is a murine J558L cell.

20. A method as recited in claim 11 wherein the immunoglobulin comprises the variable region found in a first mammalian species and comprises the constant region found

5,807,715

19

in a second mammalian species, said second mammalian species being other than the first mammalian species.

21. A method for producing a functional immunoglobulin comprising a heavy chain and a light chain which comprises the steps of:

- (a) maintaining in a nutrient medium a transformed mammalian lymphocytic cell, said cell having been transfected with a first DNA molecule coding for a first chain of the immunoglobulin and a second DNA molecule coding for a second chain of the immunoglobulin, said second chain being a chain other than the first chain and said first and second chains being either the heavy chain or the light chain;
- (b) expressing from said cell the heavy chain and the light chain functionally assembled together to form said immunoglobulin which is then secreted in a form capable of binding antigen; and
- (c) recovering said immunoglobulin wherein prior to being transfected, the cell does not express a functional immunoglobulin capable of specifically binding antigen.

22. A method as recited in claim 21 wherein the cell is transfected via protoplast fusion.

23. A method as recited in claim 21 wherein the cell is transfected via calcium phosphate precipitation.

24. A method as recited in claim 21 wherein the cell is a myeloma cell.

25. A method as recited in claim 24 wherein the cell is a murine myeloma cell.

26. A method as recited in claim 21 wherein the cell does not endogenously produce any immunoglobulin chains.

27. A method as recited in claim 26 wherein the cell is a murine P₃ cell.

28. A method as recited in claim 21 wherein prior to being transfected the cell endogenously produces an immunoglobulin light chain or an immunoglobulin heavy chain, but not both.

29. A method as recited in claim 28 wherein the cell is a murine J558L cell.

30. A method as recited in claim 21 wherein the immunoglobulin comprises the variable region found in a first mammalian source and comprises the constant region found in a second mammalian species, said second mammalian species being other than the first mammalian species.

31. A method for producing a functional antigen-binding protein comprising

- i) a first chain comprising an immunoglobulin heavy chain variable domain and an immunoglobulin heavy chain constant domain and
- ii) a second chain comprising an immunoglobulin light chain variable domain and an immunoglobulin light chain constant domain,

wherein the method comprises the steps of:

- (a) transfecting a transformed mammalian lymphocytic cell with a first DNA molecule coding for the first chain of the protein;
- (b) transfecting the cell with a second DNA molecule, said second DNA molecule coding for the second chain of the protein; and
- (c) maintaining the cell in a nutrient medium, so that the cell expresses the first and second DNA molecules and the resultant chains are intracellularly assembled together to form the protein which is then secreted in a form capable of specifically binding to antigen

wherein prior to step (a) the cell does not express a functional antigen-binding protein.

20

32. A method as recited in claim 31 wherein prior to step (a) the cell does not endogenously produce any immunoglobulin chains.

33. A method as recited in claim 31 wherein prior to step (a) the cell endogenously produces an immunoglobulin light chain or an immunoglobulin heavy chain, but not both.

34. A method as recited in claim 31 wherein the first chain comprises a constant region.

35. A method as recited in claim 31 wherein the heavy and light chain variable domains are from a first mammalian species and the heavy and light chain constant domains are from a second mammalian species, said second mammalian species being other than the first mammalian species.

36. A method as recited in claim 34 wherein the heavy and light chain variable domains are from a first mammalian species and the heavy and light chain constant regions are from a second mammalian species, said second mammalian species being other than the first mammalian species.

37. A method for producing a functional antigen-binding protein comprising

- i) a first chain comprising an immunoglobulin heavy chain variable domain and an immunoglobulin heavy chain constant domain and
- ii) a second chain comprising an immunoglobulin light chain variable domain and an immunoglobulin light chain constant domain,

wherein the method comprises the steps of:

- (a) transfecting a transformed mammalian lymphocytic cell with a plasmid comprising a first DNA molecule coding for the first chain of the protein and a second DNA molecule coding for the second chain of the protein; and
- (b) maintaining the cell in a nutrient medium so that the cell expresses said first DNA molecule and said second DNA molecule and the resultant chains are intracellularly assembled together to form the protein which is then secreted in a form capable of specifically binding to antigen

wherein prior to step (a) the cell does not express a functional immunoglobulin capable of specifically binding antigen.

38. A method as recited in claim 37 wherein prior to step (a) the cell does not endogenously produce any immunoglobulin chains.

39. A method as recited in claim 37 wherein prior to step (a) the cell endogenously produces an immunoglobulin light chain or an immunoglobulin heavy chain, but not both.

40. A method as recited in claim 37 wherein the first chain comprises a constant region.

41. A method as recited in claim 39 wherein the antigen-binding protein comprises the heavy and light chain variable domains are from a first mammalian species and comprises the heavy and light chain constant domains are from a second mammalian species, said second mammalian species being other than the first mammalian species.

42. A method as recited in claim 40 wherein the antigen-binding protein comprises the heavy and light chain variable domains are from a first mammalian species and comprises the heavy and light chain constant regions are from a second mammalian species, said second mammalian species being other than the first mammalian species.

43. A method for producing a functional antigen-binding protein comprising

- i) a first chain comprising an immunoglobulin heavy chain variable domain and an immunoglobulin heavy chain constant domain and
- ii) a second chain comprising an immunoglobulin light chain variable domain and an immunoglobulin light chain constant domain,

5,807,715

21

wherein the method comprises the steps of:

- (a) maintaining in a nutrient medium a transformed mammalian lymphocytic cell, said cell having been transfected with a first DNA molecule coding for the first chain of the protein and a second DNA molecule coding for the second chain of the protein;
- (b) expressing from said cell the first and second chains functionally assembled together to form said protein which is then secreted in a form capable of binding antigen; and
- (c) recovering said antigen-binding protein, wherein prior to being transfected, the cell does not express a functional immunoglobulin capable of specifically binding antigen.

44. A method as recited in claim 43 wherein prior to step (a) the cell does not endogenously produce any immunoglobulin chains.

45. A method as recited in claim 43 wherein prior to step (a) the cell endogenously produces an immunoglobulin light chain or an immunoglobulin heavy chain, but not both.

46. A method as recited in claim 43 wherein the first chain comprises a constant region.

47. A method as recited in claim 43 wherein the heavy and light chain variable domains are from a first mammalian species and the heavy and light chain constant domains are from a second mammalian species, said second mammalian species being other than the first mammalian species.

48. A method as recited in claim 46 wherein the heavy and light chain variable domains are from a first mammalian species and the heavy and light chain constant regions are from a second mammalian species, said second mammalian species being other than the first mammalian species.

49. A transformed mammalian lymphocytic cell producing a functional antigen-binding protein comprising

- i) a first chain comprising an immunoglobulin heavy chain variable domain and an immunoglobulin heavy chain constant domain and
- ii) a second chain comprising an immunoglobulin light chain variable domain and an immunoglobulin light chain constant domain,

wherein the transformed mammalian lymphocytic cell comprises:

- (a) a first exogenous DNA molecule coding for the first chain of the protein; and
- (b) a second exogenous DNA molecule, said second DNA molecule coding for the second chain of the protein;

wherein without the exogenous DNA molecules the cell does not express a functional antigen-binding protein.

50. A transformed mammalian lymphocytic cell as recited in claim 49 wherein without the exogenous DNA molecules the cell does not endogenously produce any immunoglobulin chains.

51. A transformed mammalian lymphocytic cell as recited in claim 49 wherein without the exogenous DNA molecules the cell endogenously produces an immunoglobulin light chain or an immunoglobulin heavy chain, but not both.

52. A transformed mammalian lymphocytic cell as recited in claim 49 wherein the first chain comprises a constant region.

22

53. A transformed mammalian lymphocytic cell as recited in claim 49 wherein the heavy and light chain variable domains are from a first mammalian species and the heavy and light chain constant domains are from a second mammalian species, said second mammalian species being other than the first mammalian species.

54. A transformed mammalian lymphocytic cell as recited in claim 52 wherein the heavy and light chain variable domains are from a first mammalian species and the heavy and light chain constant regions are from a second mammalian species, said second mammalian species being other than the first mammalian species.

55. A transformed mammalian lymphocytic cell producing a functional antigen-binding protein comprising

- i) a first chain comprising an immunoglobulin heavy chain variable domain and an immunoglobulin heavy chain constant domain and
- ii) a second chain comprising an immunoglobulin light chain variable domain and an immunoglobulin light chain constant domain,

wherein the transformed mammalian lymphocytic cell comprises:

- a) a plasmid comprising a first exogenous DNA molecule coding for the first chain of the protein and a second exogenous DNA molecule coding for the second chain of the protein; and

wherein without the exogenous DNA molecules the cell does not express a functional antigen-binding protein.

56. A transformed mammalian lymphocytic cell as recited in claim 55 wherein without the exogenous DNA molecules the cell does not endogenously produce any immunoglobulin chains.

57. A transformed mammalian lymphocytic cell as recited in claim 55 wherein without the exogenous DNA molecules the cell endogenously produces an immunoglobulin light chain or an immunoglobulin heavy chain, but not both.

58. A transformed mammalian lymphocytic cell as recited in claim 55 wherein the first chain comprises a constant region.

59. A transformed mammalian lymphocytic cell as recited in claim 55 wherein the heavy and light chain variable domains are from a first mammalian species and the heavy and light chain constant domains are from a second mammalian species, said second mammalian species being other than the first mammalian species.

60. A transformed mammalian lymphocytic cell as recited in claim 58 wherein the heavy and light chain variable domains are from a first mammalian species and the heavy and light chain constant regions are from a second mammalian species, said second mammalian species being other than the first mammalian species.

61. A transformed mammalian lymphocytic cell produced by steps (a) and (b) of claim 31.

62. A transformed mammalian lymphocytic cell produced by step (a) of claim 37.

* * * * *